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(54) Title: ELEVEN HIGHLY INFORMATIVE MICROSATELLITE REPEAT POLYMORPHIC DNA MARKERS

(57) Abstract

The invention relates to polymorphic markers (two tetranucleotide, one dinucleotide repeat polymorphisms), 27 markers characterized by primer pairs 1A-27A, and eleven markers characterized by primer pairs 1B-11B that are useful for human individualization. Applications are in forensic medicine and for paternity and prenatal screening as well as genetic mapping. These markers are characterized by sets of oligonucleotide primers according to the invention useful in PCR amplification and DNA segment resolution. The invention further relates to an assay for measuring the subtle differences in genetic material regarding an added or omitted set of dinucleotide or tetranucleotide repeat polymorphisms which comprises obtaining an amount of nucleotide segments effective for testing, amplifying the segments by the PCR procedure using at least one primer nucleotide sequence according to the present invention, resolving the amplified segments using gel electrophoresis, and comparing the resolved segments by autoradiography to observe the differences in migration patterns due to structural differences. The assay according to the invention is easy to perform and results can be obtained within 24 hours. It is not uncommon for results to be available within 3-4 hours. Accordingly, the invention also relates to an improved PCR procedure and a PCR assay kit which comprise nucleotides according to the invention.

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ELEVEN HIGHLY INFORMATIVE MICROSATELLITE REPEAT POLYMORPHIC DNA MARKERS

This is a continuation-in-part of U.S. Application 07/922,723, filed July 31, 1992, which is a continuation-in-part of U.S. Application 07/799,828, filed November 27, 1991, which is a continuation-in-part of U.S. Application 07/707,501, filed May 29, 1991.

Technical Field

This application relates to genetic testing with polymorphic DNA markers having repeat sequences to provide a rapid and convenient high resolution process for distinguishing target nucleic acid segments on the basis of nucleotide differences according to human individualization wherein the nucleic acid segments differ in size.

Background Art

The science of genetics has taken a keen interest in the identification of human individualization and genetic relationships between individuals. Each individual has hereditary material (DNA, "nucleotides") which is unique to that individual and hereditary material which is related to that of others. Procedures have been developed which are based on identification and characterization of changes in DNAs, which are changes in DNA (DNA polymorphisms) due to nucleotide substitution, insertion, or deletion within the chains of DNAs.

In the field of forensic medicine, for example, there is a keen interest in such polymorphisms for identification purposes. Forensic geneticist have

developed many techniques to compare homologous segments of DNA to determine if the segments are identical or if they differ in one or more nucleotides. Practical applications of these techniques relate to fields other than forensic medicine, for example, genetic disease diagnosis and human genome mapping.

At the present time in this art, the most accurate and informative way to compare DNA segments requires a method which provides the complete nucleotide sequence for each DNA segment. Particular techniques have been developed for determining actual sequences in order to study mutation in human genes. See, for example, Proc. Natl. Acad. Sci. U.S.A. 85, 544-548 (1988) and Nature 330, 384-386 (1987). However, because of the extensive amounts of time and high costs to determine, interpret, and compare sequence information, presently it is not practical to use extensive sequencing for compare more than just a few DNA segments.

In genetic mapping, the most frequently used screening for DNA polymorphisms arising from mutations consist of digesting the DNA strand with restriction endonucleases and analyzing the resulting fragments by means of Southern blots. See Am. J. Hum. Genet. 32, 314-331 (1980) or Sci. Am. 258, 40-48 (1988). Since mutations often occur randomly they may affect the recognition sequence of the endonuclease and preclude the enzymatic cleavage at that cite. Restriction fragment length polymorphism mappings (RFLPS) are based on changes at the restriction site. They are accurate but not very informative (PIC [0.3). The major problem with RFLPs is the inability of a test to detect changes that do not affect cleavage with a restriction endonuclease. As in many of the test

methods in the DNA art, the methods used to detect RFLPs are very labor intensive and expensive, especially the techniques which includes Southern blot analysis.

Another technique for detecting specific mutations in particular DNA segment involves hybridizing DNA segments which are being analyzed (target DNA) with a complimentary, labeled oligonucleotide probe. See Nucl. Acids Res. 9, 879-894 (1981). Since DNA duplexes containing even a single base pair mismatch exhibit high thermal instability, the differential melting temperature can be used to distinguish target DNAs that are perfectly complimentary to the probe from target DNAs that only differ by a single nucleotide. This method has been adapted to detect the presence or absence of a specific restriction site, U.S. Patent No. 4,683,194. The method involves using an end-labeled oligonucleotide probe spanning a restriction site which is hybridized to a target DNA. The hybridized duplex of DNA is then incubated with the restriction enzyme appropriate for that site. Reformed restriction sites will be cleaved by digestion in the pair of duplexes between the probe and target by using the restriction endonuclease. The specific restriction site is present in the target DNA if shortened probe molecules are detected.

Another process for studying differences in DNA structure is the primer extension process which consists of hybridizing a labeled oligonucleotide primer to a template RNA or DNA and then using a DNA polymerase and deoxynucleoside triphosphates to extend the primer to the 5′ end of the template. Resolution of the labeled primer extension product is then done by fractionating on the basis of size, e.g., by

electrophoresis via a denaturing polyacrylamide gel. This process is often used to compare homologous DNA segments and to detect differences due to nucleotide insertion or deletion. Differences due to nucleotide substitution are not detected since size is the sole criterion used to characterize the primer extension product.

Another process exploits the fact that the incorporation of some nucleotide analogs into DNA causes an incremental shift of mobility when the DNA is subjected to a size fractionation process, such as electrophoresis. Nucleotide analogs can be used to identify changes since they can cause an electrophoretic mobility shift. See, U.S. Patent 4,879,214.

Unfortunately, the above techniques used for identification of polymorphisms are either not very informative or take a long period of time to perform. For example, techniques which identify changes in individual nucleotides on a particular DNA strand often take at least three to four days to perform. Accordingly, such tests are very labor intensive and expensive to perform.

Further, subtle genetic differences among related individuals regarding nucleotides which are substituted in the DNA chains are difficult to detect. VNTR's or Jeffrey's probes (which the FBI is using to test and identify DNA chains) are very informative but labor intensive, in distinction to microsatellites as our which are equally informative PCR based polymormismic.

The use of certain nucleotide repeat polymorphisms for identifying or comparing DNA segments have been described by Weber & May 89 Am Hum Genet 44:388, Litt & Luthy '89 Am) Hum Genet 44:397). However the particular polymorphism genetic segments and primers

used to identify the polymorphisms (for identification and comparison purposes) of the present invention have not been previously known or suspected.

Accordingly, there a need in this art for a rapid, simple, inexpensive and accurate technique having a very high resolution value to determine relationships between individuals and differences in degree of relationships. Also, there is a need in the art for a very accurate genetic relationship test procedure which uses very small amounts of an original DNA sample, yet produces very accurate results. This is particularly true in the forensic medicine area and criminology, since often times very small samples of DNA are available for testing.

Disclosure of the Invention

An object of the present invention is to provide a fast and accurate test for measuring the subtle differences in individuals by way of genetic testing.

Another object of the invention relates to polymorphic markers that can be used for human individualization.

A further object of the invention is to provide a fast and accurate technique for measuring the subtle differences in individuals by way of genetic testing that can be applied in multiple areas, e.g., forensic screening, paternity and prenatal screening and genetic mapping.

A still further object is to provide an improved method for conducting a PCR procedure using an effective amount of a nucleotide according to the present invention and to provide an PCR assay kit

comprising an effective amount of a nucleotide according to the present invention and ancillary PCR reagents.

Brief Description of Drawings

Figure 1 relates to a nucleotide sequence according to SEQ ID NO:1.

Figure 2 relates to a nucleotide sequence according to SEQ ID NO:2.

Figure 3 relates to a nucleotide sequence according to SEQ ID NO:3.

Figure 4 relates to a nucleotide sequence according to SEQ ID NO:4.

Figure 5 relates to a nucleotide sequence according to SEQ ID NO:5.

Figure 6 relates to a nucleotide sequence according to SEQ ID NO:6.

Figure 7 relates to a nucleotide sequence according to SEQ ID NO:7.

Figure 8 relates to a nucleotide sequence according to SEQ ID NO:8.

Figure 9 relates to a nucleotide sequence according to SEQ ID NO:9.

Figure 10 relates to a nucleotide sequence according to SEQ ID NO:10.

Figure 11 relates to a nucleotide sequence according to SEQ ID NO:11.

Figure 12 relates to a nucleotide sequence according to SEQ ID NO:12.

Figure 13 relates to a nucleotide sequence according to SEQ ID NO:13.

Figure 14 relates to a nucleotide sequence according to SEQ ID NO:14.

Figure 15 relates to a nucleotide sequence according to SEQ ID NO:15.

Figure 16 relates to a nucleotide sequence according to SEQ ID NO:16.

Figure 17 relates to a nucleotide sequence according to SEQ ID NO:17.

Figure 18 relates to a nucleotide sequence according to SEQ ID NO:18.

Figure 19 relates to a nucleotide sequence according to SEQ ID NO:19.

Figure 20 relates to a nucleotide sequence according to SEQ ID NO:20.

Figure 21 relates to a nucleotide sequence according to SEQ ID NO:21.

Figure 22 relates to a nucleotide sequence according to SEQ ID NO:22.

Figure 23 relates to a nucleotide sequence according to SEQ ID NO:23.

Figure 24 relates to a nucleotide sequence according to SEQ ID NO:24.

Figure 25 relates to a nucleotide sequence according to SEQ ID NO:25.

Figure 26 relates to a nucleotide sequence according to SEQ ID NO:26.

Figure 27 relates to a nucleotide sequence according to SEQ ID NO:27.

Figure 28 relates to a nucleotide sequence according to SEQ ID NO:28.

Figure 29 relates to a nucleotide sequence according to SEQ ID NO:29.

Figure 30 relates to a nucleotide sequence according to SEQ ID NO:30.

Figure 31 relates to a nucleotide sequence according to SEQ ID NO:31.

Figure 32 relates to a nucleotide sequence according to SEQ ID NO:32.

Figure 33 relates to a nucleotide sequence according to SEQ ID NO:33.

Figure 34 relates to a nucleotide sequence according to SEQ ID NO:34.

Figure 35 relates to a nucleotide sequence according to SEQ ID NO:35.

Figure 36 relates to a nucleotide sequence according to SEQ ID NO:36.

Figure 37 relates to a nucleotide sequence according to SEQ ID NO:37.

Figure 38 relates to a nucleotide sequence according to SEQ ID NO:38.

Figure 39 relates to a nucleotide sequence according to SEQ ID NO:39.

Figure 40 relates to a nucleotide sequence according to SEQ ID NO:40.

Figure 41 relates to a nucleotide sequence according to SEQ ID NO:41.

Figure 42 relates to a nucleotide sequence according to SEQ ID NO:42.

Figure 43 relates to a nucleotide sequence according to SEQ ID NO:43.

Figure 44 relates to a nucleotide sequence according to SEQ ID NO:44.

Figure 45 relates to a nucleotide sequence according to SEQ ID NO:45.

Figure 46 relates to a nucleotide sequence according to SEQ ID NO:46.

Figure 47 relates to a nucleotide sequence according to SEQ ID NO:47.

Figure 48 relates to a nucleotide sequence according to SEQ ID NO:48.

Figure 49 relates to a nucleotide sequence according to SEQ ID NO:49.

Figure 50 relates to a nucleotide sequence according to SEQ ID NO:50.

Figure 51 relates to a nucleotide sequence according to SEQ ID NO:51.

Figure 52 relates to a nucleotide sequence according to SEQ ID NO:52.

Figure 53 relates to a nucleotide sequence according to SEQ ID NO:53.

Figure 54 relates to a nucleotide sequence according to SEQ ID NO:54.

Figure 55 relates to a nucleotide sequence according to SEQ ID NO:55.

Figure 56 relates to a nucleotide sequence according to SEQ ID NO:56.

Figure 57 relates to a nucleotide sequence according to SEQ ID NO:57.

Figure 58 relates to a nucleotide sequence according to SEQ ID NO:58.

Figure 59 relates to a nucleotide sequence according to SEQ ID NO:59.

Figure 60 relates to a nucleotide sequence according to SEQ ID NO:60.

Figure 61 relates to a nucleotide sequence according to SEQ ID NO:61.

Figure 62 relates to a nucleotide sequence according to SEQ ID NO:62.

Figure 63 relates to a nucleotide sequence according to SEQ ID NO:63.

Figure 64 relates to a nucleotide sequence according to SEQ ID NO:64.

Figure 65 relates to a nucleotide sequence according to SEQ ID NO:65.

Figure 66 relates to a nucleotide sequence according to SEQ ID NO:66.

Figure 67 relates to a nucleotide sequence according to SEQ ID NO:67.

Figure 68 relates to a nucleotide sequence according to SEQ ID NO:68.

Figure 69 relates to a nucleotide sequence according to SEQ ID NO:69.

Figure 70 relates to a nucleotide sequence according to SEQ ID NO:70.

Figure 71 relates to a nucleotide sequence according to SEQ ID NO:71.

Figure 72 relates to a nucleotide sequence according to SEQ ID NO:72.

Figure 73 relates to a nucleotide sequence according to SEQ ID NO:73.

Figure 74 relates to a nucleotide sequence according to SEQ ID NO:74.

Figure 75 relates to a nucleotide sequence according to SEQ ID NO:75.

Figure 76 relates to a nucleotide sequence according to SEQ ID NO:76.

Figure 77 relates to a nucleotide sequence according to SEQ ID NO:77.

Figure 78 relates to a nucleotide sequence according to SEQ ID NO:78.

Figure 79 relates to a nucleotide sequence according to SEQ ID NO:79.

Figure 80 relates to a nucleotide sequence according to SEQ ID NO:80.

Figure 81 relates to a nucleotide sequence according to SEQ ID NO:81.

Figure 82 relates to a nucleotide sequence according to SEQ ID NO:82.

Figure 83 relates to a nucleotide sequence according to SEQ ID NO:83.

Figure 84 relates to a nucleotide sequence according to SEQ ID NO:84.

Best Mode for Carrying out the Invention

The present invention provides a fast and accurate test for measuring subtle genetic differences in individuals by way of genetic testing. The invention further relates to polymorphic markers (two tetranucleotide and one dinucleotide repeat polymorphisms) that can be used for human individualization. The invention further relates to twenty-seven other polymorphic markers useful for human individualization. The invention still further relates to eleven other polymorphic markers and the eleven primer pairs useful for measuring the subtle genetic differences relating to the eleven polymorphic markers. Applications for the technique and markers according to the invention are for example, in forensic screening, in paternity and prenatal screening as well as in genetic mapping.

The invention relates to polymorphic markers (two tetranucleotide, one dinucleotide repeat polymorphisms, twenty-seven other unique polymorphic markers, and eleven more unique polymorphic markers) that are useful for human individualization for a forensic screen, and for paternity and prenatal screening as well as genetic mapping. The markers according to the present invention have high polymorphism information content (PIC) values. The first three markers are

characterized by sets of oligonucleotide primers as follows:

1. Set 1, PIC 0.92
    a. A nucleotide sequence according to SEQ ID NO:1
    b. A nucleotide sequence according to SEQ ID NO:2
2. Set 2, PIC 0.91
    a. A nucleotide sequence according to SEQ ID NO:3
    b. A nucleotide sequence according to SEQ ID NO:4
3. Set 3, PIC 0.92
    a. A nucleotide sequence according to SEQ ID NO:5
    b. A nucleotide sequence according to SEQ ID NO:6.

These polymorphic markers (two tetranucleotide and one dinucleotide repeat polymorphisms which are also accompanied by beginning and ending nucleotide sequences) that can be used for human individualization are further characterized by the following marker sequences.

1. A nucleotide sequence having a repeat polymorphism according to SEQ ID NO:7.

2. A nucleotide sequence having a repeat polymorphism according to SEQ ID NO:8.

3. A nucleotide sequence having a repeat polymorphism according to SEQ ID NO:9.

Since a polymorphic marker and an index locus occur as a "pair", attaching a primer oligonucleotide according to the present invention to the polymorphic marker allows PCR amplification of the segment pair. The amplified DNA segment can then be resolved by

electrophoresis and autoradiography. A resulting autoradiography can then be analyzed for its similarity to another DNA segment autoradiography. Following the PCR amplification procedure, electrophoretic motility enhancing DNA analogs may optionally be used to increase the accuracy of the electrophoresis step.

Twenty-seven other primary pair sequences for detecting unique polymorphisms are sequences according to SEQ ID NO:10 through SEQ ID NO:63. Additionally, eleven other primary pair sequences for detecting unique polymorphisms are sequences according to SEQ ID NO:64 through SEQ ID NO:73.

The described polymorphisms are useful for human sample individualization, because of their high PIC values. Since the described polymorphisms are based on the polymerase chain reaction, only minute amounts of genomic DNA are required for each test. The target sequences range from 69-260 bps in length so that high molecular weight DNA is not necessary and common problems such as shearing of DNA will have minimal impact on the performance of the assay. The assay is easy to perform and results can be obtained within 24 hours. Microsatellite repeat polymorphisms have been shown to be useful tools in DNA analysis. The 27 polymorphisms described here are original and are based on previously sequenced human genes. The eleven further polymorphisms described are original. The most commonly used technique in forensic screening is based on minisatellite markers in distinction to the PCR able microsatellites described here.

The 27 markers are characterized by sets of oligonucleotide primers as set forth in Table 1, below. The 27 pairs are indicated in Table 1 as 1A-27A, respectively. Also indicated is the locus, the

chromosomal location, the primer SEQ ID NO:, the degree of heterozygousness, the PIC value, the size, the repeat sequence and the number of alleles.

The additional eleven markers are characterized by sets of oligonucleotide primers as set forth in Table 2, below. These eleven pairs are indicated in Table 2 as 1B-11B, respectively. Also indicated is the locus, the chromosomal location, the primer SEQ ID NO:, the degree of heterozygousness, the PIC value, the size, the repeat sequence and the number of alleles.

TABLE 1

Pair #	Locus	Chromosonal Location	Primer SEQ ID NO:	Heteroz	PIC	Size	Repeat	No. of alleles
1A	Int-2	11q13	10,11	84.6%	0.79	161-177	$(TG)_5TC(TG)_{16}$	9
2A	PLA-AZ	12	12,13	73.3%	0.76	122-137	$(TTA)_{16}$	6
3A	FABP2	4q28-q31	14,15	64%	0.64	99-117	$(TTA)_{13}$	6
4A	THROO1	15q15	16,17	60%	0.58	165-181	$(CT)_{14}$	9
5A	CYARP450	15Fq21.1	18,19	91.3%	0.67	175-199	$(TTTA)_8$	5
6A	GCG	2q36-q37	20,21	88%	0.77	142-172	$(GA)_{19}$	11
7A	IL-9	5q	22,23	62.5%	0.75	127-139	$(TG)_{20}$	7
8A	CSTP1	20	24,25	61%	0.58	123-141	$(GT)_{15}$	
9A	ANKYRIN	8p11.1-21.1	26,27	54%	0.45	107-113	$(AC)_{14}$	4
10A	CD-19	16	28,29	40%	0.39	79-91	$(GT)_{17}$	7
11A	c-fms	5q33.3-34	30,31	86%	0.85	95-127	$(GT)_{26}$	10
12A	CD 8	2p12	32,33	71%	0.66	138-170	$(AC)_{14}$	7
13A	CYP2D7-8	22	34,35	80%	0.78	98-116	$(GT)_{18}$	10
14A	W 30	7q	36,37	74%	0.72			11
15A	HMG-14	21	38,39	69%	0.67	69-93	$(GT)_{19}$	10
16A	RHO	3	40,41	72%	0.68	145-169		5
17A	PFKL	21q22.3	42,43	70%	0.66	129-145	$(AC)_{16}$	7
18A	HSFLT	13q12	44,45	51%	0.49	164-186	$(TG)_{21}$	8
19A	HSMYHO1	14	46,47	66%	0.60	90-102	$(GT)_{15}$	6
20A	HSATPSY1	12p13-qter	48,49	60%	0.54	111-117	$(GT)_{11}$	4
21A	CPES PPS	15q25-qter	50,51	75%	0.70	143-163	$(ATTT)_{11}$	6
22A	DHFRP2	6	52,53	70%	0.66	157-173	$(AAAC)_7$	5
23A	CRYG1	2q34-35	54,55	68%	0.61	117-126	$(AAC)_9$	4
24A	F13A1	6p24-25	56,57	78%	0.75	180-230	$(AAAG)_7$	8
25A	TRM1	6p23-q12	58,59	54%	0.50	174-186	$(AAC)_8$	5
26A	II-D	6	60,61	81%	0.78	185-206	$(CAG)_{18}$	
27A	TH	11p15.5-p15	62,63	78%	0.75	244-260	$(TCAT)_9$	5

TABLE 2

#	Locus	Chromosomal Location	Primer SEQ ID NO:	Heteroz	PIC	Size	Repeat	No. of alleles
	ACPP	3q21-qter	64	69%	0.65	260-280	$(AAAT)_{11}$	6
			65					
	MSP	18q23-qter	66	80%	0.77	208-232	$(ATGG)_{12}$,$(TGGA)_9$	7
			67	79%	0.76	122-142	$(ATGG)_{12}$	6
	IGF1	12q23	68	53%	0.52	173-207	$(CT)_{16}$	11
			69					
	GABRB1	4p12-p13	70	72%	0.68	91-99	$(AC)_{19}$	5
			71					
	MYC	8q24	72	86%	0.85	87-125	$(AT)_{23}$	15
			73					
	D3S1229		74	84%	0.83	109-127	$(AC)_{10}$	10
			75					
	D5S356		76	90%	0.89	94-132	$(AC)_{29}$	14
			77					
			78	81%	0.79	104-132	$(AC)_{22}$	13
			79					
	D3S1247		80	80%	0.77	153-173	$(AC)_{21}$	8
			81					
	D3S1246		82	82.5%	0.80	110-128	$(GT)_{20}$	10
			83					
	D9S147E		84	78%	0.75	189-201	$(GT)_{20}$	7
			85					

Also, the invention relates to a method for conducting a PCR procedure comprising using an effective amount of at least one nucleotide according to according to the invention as set forth above, wherein the nucleotide is part of a primer pair of nucleotides selected from the group of nucleotide pairs consisting of

a) a polynucleotide having the sequence as set forth in SEQ ID NO:1 and a polynucleotide having a sequence as set forth in SEQ ID NO:2;

b) a polynucleotide having the sequence as set forth in SEQ ID NO:3 and a polynucleotide having the sequence as set forth in SEQ ID NO:4;

c) a polynucleotide having the sequence as set forth in SEQ ID NO:5 and a polynucleotide having the sequence as set forth in SEQ ID NO:6;

d) a polynucleotide having the sequence as set forth in SEQ ID NO:10 and a polynucleotide having the sequence as set forth in SEQ ID NO:11;

e) a polynucleotide having the sequence as set forth in SEQ ID NO:12 and a polynucleotide having the sequence as set forth in SEQ ID NO:13;

f) a polynucleotide having the sequence as set forth in SEQ ID NO:14 and a polynucleotide having the sequence as set forth in SEQ ID NO:15;

g) a polynucleotide having the sequence as set forth in SEQ ID NO:16 and a polynucleotide having the sequence as set forth in SEQ ID NO:17;

h) a polynucleotide having the sequence as set forth in SEQ ID NO:18 and a polynucleotide having the sequence as set forth in SEQ ID NO:19;

i) a polynucleotide having the sequence as set forth in SEQ ID NO:20 and a polynucleotide having the sequence as set forth in SEQ ID NO:21;

j) a polynucleotide having the sequence as set forth in SEQ ID NO:22 and a polynucleotide having the sequence as set forth in SEQ ID NO:23;

k) a polynucleotide having the sequence as set forth in SEQ ID NO:24 and a polynucleotide having the sequence as set forth in SEQ ID NO:25;

l) a polynucleotide having the sequence as set forth in SEQ ID NO:26 and a polynucleotide having the sequence as set forth in SEQ ID NO:27;

m) a polynucleotide having the sequence as set forth in SEQ ID NO:28 and a polynucleotide having the sequence as set forth in SEQ ID NO:29;

n) a polynucleotide having the sequence as set forth in SEQ ID NO:30 and a polynucleotide having the sequence as set forth in SEQ ID NO:31;

o) a polynucleotide having the sequence as set forth in SEQ ID NO:32 and a polynucleotide having the sequence as set forth in SEQ ID NO:33;

p) a polynucleotide having the sequence as set forth in SEQ ID NO:34 and a polynucleotide having the sequence as set forth in SEQ ID NO:35;

q) a polynucleotide having the sequence as set forth in SEQ ID NO:36 and a polynucleotide having the sequence as set forth in SEQ ID NO:37;

r) a polynucleotide having the sequence as set forth in SEQ ID NO:38 and a polynucleotide having the sequence as set forth in SEQ ID NO:39;

s) a polynucleotide having the sequence as set forth in SEQ ID NO:40 and a polynucleotide having the sequence as set forth in SEQ ID NO:41;

t) a polynucleotide having the sequence as set forth in SEQ ID NO:42 and a polynucleotide having the sequence as set forth in SEQ ID NO:43;

u) a polynucleotide having the sequence as set forth in SEQ ID NO:44 and a polynucleotide having the sequence as set forth in SEQ ID NO:45;

v) a polynucleotide having the sequence as set forth in SEQ ID NO:46 and a polynucleotide having the sequence as set forth in SEQ ID NO:47;

w) a polynucleotide having the sequence as set forth in SEQ ID NO:48 and a polynucleotide having the sequence as set forth in SEQ ID NO:49;

x) a polynucleotide having the sequence as set forth in SEQ ID NO:50 and a polynucleotide having the sequence as set forth in SEQ ID NO:51;

y) a polynucleotide having the sequence as set forth in SEQ ID NO:52 and a polynucleotide having the sequence as set forth in SEQ ID NO:53;

z) a polynucleotide having the sequence as set forth in SEQ ID NO:54 and a polynucleotide having the sequence as set forth in SEQ ID NO:55;

aa) a polynucleotide having the sequence as set forth in SEQ ID NO:56 and a polynucleotide having the sequence as set forth in SEQ ID NO:57;

bb) a polynucleotide having the sequence as set forth in SEQ ID NO:58 and a polynucleotide having the sequence as set forth in SEQ ID NO:59;

cc) a polynucleotide sequence having the sequence as set forth in SEQ ID NO:60 and a polynucleotide sequence as set forth in SEQ ID NO:61;

dd) a polynucleotide having the sequence as set forth in SEQ ID NO:62 and a polynucleotide having the sequence as set forth in SEQ ID NO:63.

ee) a polynucleotide having the sequence as set forth in SEQ ID NO:64 and a polynucleotide having the sequence as set forth in SEQ ID NO:65;

ff) a polynucleotide having the sequence as set forth in SEQ ID NO:66 and a polynucleotide having the sequence as set forth in SEQ ID NO:67;

gg) a polynucleotide having the sequence as set forth in SEQ ID NO:68 and a polynucleotide having the sequence as set forth in SEQ ID NO:69;

hh) a polynucleotide having the sequence as set forth in SEQ ID NO:70 and a polynucleotide having the sequence as set forth in SEQ ID NO:71;

ii) a polynucleotide having the sequence as set forth in SEQ ID NO:72 and a polynucleotide having the sequence as set forth in SEQ ID NO:73;

jj) a polynucleotide having the sequence as set forth in SEQ ID NO:74 and a polynucleotide having the sequence as set forth in SEQ ID NO:75;

kk) a polynucleotide having the sequence as set forth in SEQ ID NO:76 and a polynucleotide having the sequence as set forth in SEQ ID NO:77;

ll) a polynucleotide having the sequence as set forth in SEQ ID NO:78 and a polynucleotide having the sequence as set forth in SEQ ID NO:79;

mm) a polynucleotide having the sequence as set forth in SEQ ID NO:80 and a polynucleotide having the sequence as set forth in SEQ ID NO:81;

nn) a polynucleotide having the sequence as set forth in SEQ ID NO:82 and a polynucleotide having the sequence as set forth in SEQ ID NO:83; and

oo) a polynucleotide having the sequence as set forth in SEQ ID NO:84 and a polynucleotide having the sequence as set forth in SEQ ID NO:85.

Therefore, the invention further relates to an assay for measuring the subtle differences in genetic material regarding an added or omitted set of dinucleotide or tetranucleotide repeat polymorphisms

selected from the group consisting of a sequence according to SEQ ID NO:7, a sequence according to SEQ ID NO:8 and a sequence according to SEQ ID NO:9, which comprises

a. obtaining nucleotide segments comprising said repeat polymorphisms in an amount effective for testing,

b. amplifying said segments by a PCR procedure using a pair of oligonucleotide primers capable of amplifying said polymorphism containing segments,

c. resolving the amplified segments using page gels electrophoresis, and

d. comparing the resolved segments by autoradiography to observe the differences in migration patterns due to length variation.

Preferably, the invention further relates to an assay for measuring the subtle differences in genetic material regarding an added or omitted set of dinucleotide or tetranucleotide repeat polymorphisms selected from the group consisting of a sequence according to SEQ ID NO:7, a sequence according to SEQ ID NO:8 and a sequence according to SEQ ID NO:9, which comprises

a. obtaining nucleotide segments comprising said repeat polymorphisms in an amount effective for testing,

b. amplifying said segments by a PCR procedure using the pair of oligonucleotide primers selected from the group consisting of a sequence according to SEQ ID NO:1, a sequence according to SEQ ID NO:2, a sequence according to SEQ ID NO:3, a sequence according to SEQ ID NO:4, a sequence according to SEQ ID NO:5, or a sequence according to SEQ ID NO:6,

c. resolving the amplified segments using PAGE gels and electrophoresis, and

d. comparing the resolved segments by auto-radiography to observe the differences in migration patterns due to length variation.

Still further, the invention relates to an assay kit for conducting a PCR procedure comprising an effective amount of at least one nucleotide having a sequence according to the invention as set forth above, wherein the nucleotide is part of a primer pair of polynucleotides selected from the group of polynucleotide pairs consisting of

a) a polynucleotide having the sequence as set forth in SEQ ID NO:1 and a polynucleotide having the sequence as set forth in SEQ ID NO:2;

b) a polynucleotide having the sequence as set forth in SEQ ID NO:3 and a polynucleotide having the sequence as set forth in SEQ ID NO:4;

c) a polynucleotide having the sequence as set forth in SEQ ID NO:5 and a polynucleotide having the sequence as set forth in SEQ ID NO:6,

d) a polynucleotide having the sequence as set forth in SEQ ID NO:10 and a polynucleotide having the sequence as set forth in SEQ ID NO:11;

e) a polynucleotide having the sequence as set forth in SEQ ID NO:12 and a polynucleotide having the sequence as set forth in SEQ ID NO:13;

f) a polynucleotide having the sequence as set forth in SEQ ID NO:14 and a polynucleotide having the sequence as set forth in SEQ ID NO:15;

g) a polynucleotide having the sequence as set forth in SEQ ID NO:16 and a polynucleotide having the sequence as set forth in SEQ ID NO:17;

h) a polynucleotide having the sequence as set forth in SEQ ID NO:18 and a polynucleotide having the sequence as set forth in SEQ ID NO:19;

i) a polynucleotide having the sequence as set forth in SEQ ID NO:20 and a polynucleotide having the sequence as set forth in SEQ ID NO:21;

j) a polynucleotide having the sequence as set forth in SEQ ID NO:22 and a polynucleotide having the sequence as set forth in SEQ ID NO:23;

k) a polynucleotide having the sequence as set forth in SEQ ID NO:24 and a polynucleotide having the sequence as set forth in SEQ ID NO:25;

l) a polynucleotide having the sequence as set forth in SEQ ID NO:26 and a polynucleotide having the sequence as set forth in SEQ ID NO:27;

m) a polynucleotide having the sequence as set forth in SEQ ID NO:28 and a polynucleotide having the sequence as set forth in SEQ ID NO:29;

n) a polynucleotide having the sequence as set forth in SEQ ID NO:30 and a polynucleotide having the sequence as set forth in SEQ ID NO:31;

o) a polynucleotide having the sequence as set forth in SEQ ID NO:32 and a polynucleotide having the sequence as set forth in SEQ ID NO:33;

p) a polynucleotide having the sequence as set forth in SEQ ID NO:34 and a polynucleotide having the sequence as set forth in SEQ ID NO:35;

q) a polynucleotide having the sequence as set forth in SEQ ID NO:36 and a polynucleotide having the sequence as set forth in SEQ ID NO:37;

r) a polynucleotide having the sequence as set forth in SEQ ID NO:38 and a polynucleotide having the sequence as set forth in SEQ ID NO:39;

s) a polynucleotide having the sequence as set forth in SEQ ID NO:40 and a polynucleotide having the sequence as set forth in SEQ ID NO:41;

t) a polynucleotide having the sequence as set forth in SEQ ID NO:42 and a polynucleotide having the sequence as set forth in SEQ ID NO:43;

u) a polynucleotide having the sequence as set forth in SEQ ID NO:44 and a polynucleotide having the sequence as set forth in SEQ ID NO:45;

v) a polynucleotide having the sequence as set forth in SEQ ID NO:46 and a polynucleotide having the sequence as set forth in SEQ ID NO:47;

w) a polynucleotide having the sequence as set forth in SEQ ID NO:48 and a polynucleotide having the sequence as set forth in SEQ ID NO:49;

x) a polynucleotide having the sequence as set forth in SEQ ID NO:50 and a polynucleotide having the sequence as set forth in SEQ ID NO:51;

y) a polynucleotide having the sequence as set forth in SEQ ID NO:52 and a polynucleotide having the sequence as set forth in SEQ ID NO:53;

z) a polynucleotide having the sequence as set forth in SEQ ID NO:54 and a polynucleotide having the sequence as set forth in SEQ ID NO:55;

aa) a polynucleotide having the sequence as set forth in SEQ ID NO:56 and a polynucleotide having the sequence as set forth in SEQ ID NO:57;

bb) a polynucleotide having the sequence as set forth in SEQ ID NO:58 and a polynucleotide having the sequence as set forth in SEQ ID NO:59;

cc) a polynucleotide having the sequence as set forth in SEQ ID NO:60 and a polynucleotide having the sequence as set forth in SEQ ID NO:61;

dd) a polynucleotide having the sequence as set forth in SEQ ID NO:62 and a polynucleotide having the sequence as set forth in SEQ ID NO:63;

ee) a polynucleotide having the sequence as set forth in SEQ ID NO:64 and a polynucleotide having the sequence as set forth in SEQ ID NO:65;

ff) a polynucleotide having the sequence as set forth in SEQ ID NO:66 and a polynucleotide having the sequence as set forth in SEQ ID NO:67;

gg) a polynucleotide having the sequence as set forth in SEQ ID NO:68 and a polynucleotide having the sequence as set forth in SEQ ID NO:69;

hh) a polynucleotide having the sequence as set forth in SEQ ID NO:70 and a polynucleotide having the sequence as set forth in SEQ ID NO:71; and

ii) a polynucleotide having the sequence as set forth in SEQ ID NO:72 and a polynucleotide having the sequence as set forth in SEQ ID NO:73;

jj) a polynucleotide having the sequence as set forth in SEQ ID NO:74 and a polynucleotide having the sequence as set forth in SEQ ID NO:75;

kk) a polynucleotide having the sequence as set forth in SEQ ID NO:76 and a polynucleotide having the sequence as set forth in SEQ ID NO:77;

ll) a polynucleotide having the sequence as set forth in SEQ ID NO:78 and a polynucleotide having the sequence as set forth in SEQ ID NO:79;

mm) a polynucleotide having the sequence as set forth in SEQ ID NO:80 and a polynucleotide having the sequence as set forth in SEQ ID NO:81;

nn) a polynucleotide having the sequence as set forth in SEQ ID NO:82 and a polynucleotide having the sequence as set forth in SEQ ID NO:83; and

oo) a polynucleotide having the sequence as set forth in SEQ ID NO:84 and a polynucleotide having the sequence as set forth in SEQ ID NO:85;

wherein said polynucleotide is in combination with an effective amount of ancillary PCR reagents.

Accordingly, the above described polymorphisms are useful for human sample individualization, because of their high PIC values. Since the described polymorphic systems are based on the polymerase chain reaction (PCR), only minute (40 nanograms) amounts of genomic DNA are required for each test. The target sequences range from 92 to 310 base pairs so that high molecular weight DNA is not necessary, and common problems such as shearing of DNA will have minimal impact on the performance of the assay. The assay is easy to perform and results can be obtained within 24 hours. It is not uncommon for results to be available within 3-4 hours. By comparison, the prior art methods require a number of days before results are available, usually 3-4 days are required.

Also, the polymorphism corresponding to 1A-27A as described above and characterizes by their 27 primer pairs according to SEQ ID NO:10-SEQ NO:63 are useful for human sample individualization evaluation because of their high PIC values. Additionally, the polymorphisms corresponding to 1B-11B as described above and characterizes by their eleven primer pairs according to SEQ ID NO:64-SEQ ID NO:85 are useful for human sample individualization evaluation because of their high PIC values.

Further, the assay according to the invention is able to detect very small differences in nucleotide sequences. A single omission or addition of the repeat sequence will change the mobility due to the electrical

nature and molecular weight of the target nucleotide sequence. These differences are clearly visible on the autoradiographs after electrophoresis.

Microsatellite repeat polymorphisms have been shown to be useful tools in DNA analysis. The three polymorphisms described here are original and are based on previously sequenced genes. The two tetranucleotide repeat markers described, can be scored easily since allele sizes differ by four base pairs. The most commonly used technique used in forensic screening is based on minisatellite markers, in distinction to the PCR able microsatellites described in the present invention.

The general PCR technique step is conducted generally as described in U.S. Patent No. 4,683,195 to Mullis et al and U.S. Patent No. 4,683,202 to Mullis et al, which are hereby incorporated by reference thereto. Further, electrical motility enhancing DNA analogs can optionally be used during the replication and amplification PCR procedure.

The degree of polymorphism in the genetic segments according to the present invention, which polymorphisms yield highly informative identification test results, is surprising and unexpected. The high PIC value (approximately 0.9) is totally unexpected.

Accordingly, the use of a PCR procedure and PCR primers pairs, such as those primer sequences according to SEQ ID NO:1 to SEQ ID NO:6, to detect the polymorphism DNA segment according to the present invention yields excellent results. Further use of primer sequences corresponding to SEQ ID NO:10 through SEQ ID NO:63 or SEQ ID NO:64 through SEQ ID NO:85 to detect the polymorphism yields excellent results. Such results are sufficiently accurate and informative to

accurately identify DNA segments and determine degrees of relationship between DNA segments of individuals.

Moreover, conducting three sets of PCR procedures on the same DNA segment samples while using a different PCR primer pair according to the present invention for each of the three procedures yields extraordinarily accurate and informative test results. Comparison of the three sets of test results data provides extremely accurate DNA segment identification.

The following examples are provided to more specifically describe the invention which is not limited to the following examples.

The described oligonucleotide primers are used to amplify the target sequences using PCR, under the following conditions:

Example 1

The samples are of DNA are prepared as follows.

60ng of genomic DNA are used as template for PCR with 80ng of each oligonucleotide primer, 0.6 units of Taq Polymerase 50mM KCL, 10mM Tris (pH 8.3), 1.5mM $MgCl_2$, 0.01% gelatin, 200uM of each dGTP, dATP, dTTP, 2.5uM dCTP and 10 microcuries of alpha P32 dCTP., in a final reaction volume of 15 microliters. The samples are overlaid with 15 microliters of mineral oil to prevent evaporation.

Example 2

PCR is performed for each of the samples and primers described in Example 1, above.

PCR is performed in a Techne MW-1 microplate thermocycler under the following conditions denaturation of 94 degrees C for 1.4 min., annealing at 55 degrees C for 2 min., and extension at 72 degrees C for 2 min. The cycle is repeated 30 times with a final extension at 72 degrees C for 10 min.

Example 3

The amplified DNA segments from each of the samples described in Example 2 above are resolved by electrophoresis as follows.

Two microliters of each PCR reaction mixture sample are electrophoresed on a 6% PAGE sequencing gel and visualized by autoradiography. Exposure times for the autoradiography range from 3-16 hours.

The foregoing descriiption of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying current knowledge, readily modify and/or adapt for various applications such specific embodiments without departing from the generic concept and therefore such adaptations are intended to be comprehended within the meaning and range of equivalents of a disclosed embodiment. It is to be understood that the phraseology or terminology employed herein is for the purposes of description only and not of limitation.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Drs. Mihael H. Polymeropoulos and Carl R. Merril

(ii) TITLE OF INVENTION: ELEVEN HIGHLY INFORMATIVE REPEAT POLYMORPHIC DNA MARKERS

(iii) NUMBER OF SEQUENCES: 85

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Lowe, Price, LeBlanc & Becker
(B) STREET: Suite 300, 99 Canal Center Plaza
(C) CITY: Alexandria
(D) STATE: Virginia
(E) COUNTRY: USA
(F) ZIP: 22314

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: DOS Text File

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: J.G. Mullins
(B) REGISTRATION NUMBER: 33073
(C) reference/docket number: 717081C

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 703 684 1111

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AATCTGGGCG ACAAGAGTGA 20

(3) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ACATCTCCCC TACCGCTATA 20

(4) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TCCAGCCTCG GAGACAGAAT 20

(5) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AGTCCTTTCT CCAGAGCAGG T 21

(6) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GCCAGTGATG CTAAAGGTTG 20

(7) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

AACATACGTG GCTCTATGCA 20

(8) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 291
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

AATCTGGGCG ACAAGAGTGA AACTCCGTCA AAAGAAAGAA AGAAAGAGAC AAAGAGAGTT 60

AGAAAGAAAG AAAGAGAGAG AGAGAGAAAG GAAGGAAGGA AGAAAAAGAA AGAAAAAGAA 120

AGAAAGAGAA AGAAAGAAAG AGAAAGAAAG AAAGAAAGAA AGAAAGAAAG AAAGAAAGAA 180

AGAAAGAAAA AGAAAGAAAG AAAGAAAGAA AGAAAGAAAG AAAGAAAGAA AGAAAGAAAG 240

AAAGAAAGGA AGGAAAGAAA GAGCAAGTTA CTATAGCGGT AGGGGAGATG T 291

(9) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 128
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GCCAGTGATG CTAAAGGTTG TATTGCATAT ATACATATAT ATATATATAT ATATATATAT 60

ATATATATAT ATATATATAT ATATATATAT TTTAATTTGA TAGTATTGTG CATAGAGCCA 120

CGTATGTT 128

(10) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 243
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TCCAGCCTCG GAGACAGAAT GAGACTCCAT CAAAAACAAG AAAGAAAGAA AGACAAAGAG 60

AGAAAGAAAG AAAGAAAGAA AGAAAGAAAG AGAGAGAGAG AGAGAGAGAG AGAAAGAAAG 120

AAAGAAAGAA AGAAAGAAAG AAAGAAAGAA AGAAAGAAAG AAAGAAAGAA GGAAAGAAAG 180

AAAGGAAACT AAAATAACTA AATAACTGAG TAGCACCACA CCACCTGCTC TGGAGAAAGG 240

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(11) INFORMATION FOR SEQ ID NO:10:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TTTCTGGGTG TGTCTGAAT 19

(12) INFORMATION FOR SEQ ID NO:11:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ACACAGTTGC TCTAAAGGGT 20

(13) INFORMATION FOR SEQ ID NO:12:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CTAGGTTGTA AGCTCCATGA 20

(14) INFORMATION FOR SEQ ID NO:13:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TTGAGCACTT ACTCTGTGCC 20

(15) INFORMATION FOR SEQ ID NO:14:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

AACTCAGAAC AGTGCCTGAC 20

(16) INFORMATION FOR SEQ ID NO:15:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

ATTTCCCTCA AGGCTCCAGG T 21

(17) INFORMATION FOR SEQ ID NO:16:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CTGATCTTGC TCACCTTCGA 20

(18) INFORMATION FOR SEQ ID NO:17:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GCGTTTGCTG AAATGAAGGA 20

(19) INFORMATION FOR SEQ ID NO:18:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GCAGGTACTT AGTTAGCTAC 20

(20) INFORMATION FOR SEQ ID NO:19:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TTACAGTGAG CCAAGGTCGT 20

(21) INFORMATION FOR SEQ ID NO:20:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

TTTGTCTGGA TAGACTGGAG 20

(22) INFORMATION FOR SEQ ID NO:21:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CCATCTTCCT GTGGCTGTA 19

(23) INFORMATION FOR SEQ ID NO:22:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CTAATGCAGA GATTTAGGGC 20

(24) INFORMATION FOR SEQ ID NO:23:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GTGGTGTAAA GACTGCATAG 20

(25) INFORMATION FOR SEQ ID NO:24:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

ATGTGACTGA TGTGGGTCAG 20

(26) INFORMATION FOR SEQ ID NO:25:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CATCTGCACT CATGCTCCAT 20

(27) INFORMATION FOR SEQ ID NO:26:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

TCCCAGATCG CTCTACATGA 20

(28) INFORMATION FOR SEQ ID NO:27:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CACAGCTTCA GAAGTCACAG 19

(29) INFORMATION FOR SEQ ID NO:28:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GAGCAATGTT GCTTAGGATG 20

(30) INFORMATION FOR SEQ ID NO:29:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

TGGAAGTGTC ACTGGCATGT 20

(31) INFORMATION FOR SEQ ID NO:30:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

TGTGTCCAGC CTTAGTGTGC A 21

(32) INFORMATION FOR SEQ ID NO:31:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

TCATCACTTC CAGAATGTGC 20

(33) INFORMATION FOR SEQ ID NO:32:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

ACTGCCTCAT CCAGTTTCAG 20

(34) INFORMATION FOR SEQ ID NO:33:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GAGCAGGCAC TTGTTAGATG 20

(35) INFORMATION FOR SEQ ID NO:34:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

CCTCTTGGCT CTAACAGCAA 20

(36) INFORMATION FOR SEQ ID NO:35:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

AGCAAGACCC TGTCTCAAGA 20

(37) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

CAAGGCCCAT CTTCAGTAGA 20

(38) INFORMATION FOR SEQ ID NO:37:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

CCTTCTCACT CCTTTACTAG 20

(39) INFORMATION FOR SEQ ID NO:38:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

GAAGACTGAG GAGGTCAGAA 20

(40) INFORMATION FOR SEQ ID NO:39:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

CTACTGTTCA GAGTCAAAGC 20

(41) INFORMATION FOR SEQ ID NO:40:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

TGCCCCACAT TAGGATGCAT 20

(42) INFORMATION FOR SEQ ID NO:41:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

AGGGACACGA ATCAGATCAG 20

(43) INFORMATION FOR SEQ ID NO:42:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

GTGGTACCTC ATTGTGGCTA 20

(44) INFORMATION FOR SEQ ID NO:43:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

AGGCATCCTT GTGCTGACAT 20

(45) INFORMATION FOR SEQ ID NO:44:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

TTTGGCCGAC AGTGGTGTAA 20

(46) INFORMATION FOR SEQ ID NO:45:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

AGGACCAAAC CATGTCTGTC 20

(47) INFORMATION FOR SEQ ID NO:46:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

CTGCATCTGA GCATATGGGA 20

(48) INFORMATION FOR SEQ ID NO:47:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

CATTCAGACT ATGCAGGCTT 20

(49) INFORMATION FOR SEQ ID NO:48:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

CTGGGACTAC TGGCACATG 19

(50) INFORMATION FOR SEQ ID NO:49:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

GGCAACGTGG TGAAACCTT 19

(51) INFORMATION FOR SEQ ID NO:50:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

GGAAGATGGA GTGGCTGTTA 20

(52) INFORMATION FOR SEQ ID NO:51:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

CTCCAGCCTG GCGAAAGAAT 20

(53) INFORMATION FOR SEQ ID NO:52:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

GTAAGACTTT TGGAGCCATT

(54) INFORMATION FOR SEQ ID NO:53:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

TTCAGGGAGA ATGAGATGGG 20

(55) INFORMATION FOR SEQ ID NO:54:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

GACAGAGTGA GACTCCATCT 20

(56) INFORMATION FOR SEQ ID NO:55:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

GATCCTATCT TCTCAGGAGG 20

(57) INFORMATION FOR SEQ ID NO:56:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

GAGGTTGCAC TCCAGCCTTT 20

(58) INFORMATION FOR SEQ ID NO:57:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

ATGCCATGCA GATTAGAAA 19

(59) INFORMATION FOR SEQ ID NO:58:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

GGAAAGAAAC AGTGAAAGA 19

(60) INFORMATION FOR SEQ ID NO:59:
(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

ATCCATCGAC CTCTGGGTTA 20

(61) INFORMATION FOR SEQ ID NO:60:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

GACCCCACAG CCTATTCAGA 20

(62) INFORMATION FOR SEQ ID NO:61:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

TTGACTGCTG AACGGCTGCA 20

(63) INFORMATION FOR SEQ ID NO:62:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

CAGCTGCCCT AGTCAGCAC 19

(64) INFORMATION FOR SEQ ID NO:63:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

GCTTCCGAGT GCAGGTCACA 20

(65) INFORMATION FOR SEQ ID NO:64:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

GGGCAACATG GTGAAACCTT 20

(66) INFORMATION FOR SEQ ID NO:65:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

CCTAGCCTAT ACTTCCTTTC 20

(67) INFORMATION FOR SEQ ID NO:66:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

GGACCTCGTG AATTACAATC 20

(68) INFORMATION FOR SEQ ID NO:67:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

ATTTACCTAC CTGTTCATCC 20

(69) INFORMATION FOR SEQ ID NO:68:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

TTGTGTCAAC TGCTGATATG 20

(70) INFORMATION FOR SEQ ID NO:69:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

AACCAAAACA TCATTCCCTA 20

(71) INFORMATION FOR SEQ ID NO:70:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

CGTAAGCGTG CACTATACCC T 21

(72) INFORMATION FOR SEQ ID NO:71:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

CTGAGGATTC ATCCACCTG 19

(73) INFORMATION FOR SEQ ID NO:72:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

CCTGAGTAGC TGTTAAGGGA 20

(74) INFORMATION FOR SEQ ID NO:73:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

GCACATGTAC CCTAGAACTT 20

(75) INFORMATION FOR SEQ ID NO:74:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

AATCTGAACA GTAATGAAGG 20

(76) INFORMATION FOR SEQ ID NO:75:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

CATTCTGATA CATTACAGTC 20

(77) INFORMATION FOR SEQ ID NO:76:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

ATTCCGAGTG ATTTCAGAGA 20

(78) INFORMATION FOR SEQ ID NO:77:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

TGCTGGTTCA CAGAGCCCTG 20

(79) INFORMATION FOR SEQ ID NO:78:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

TAGCAGTTCA CAGAGCCCTG 20

(80) INFORMATION FOR SEQ ID NO:79:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

GTAATTAACA AACCGAGCTG 20

(81) INFORMATION FOR SEQ ID NO:80:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCIPTION: SEQ ID NO:80:

AGTATCTGTG CACTGTCTGG 20

(82) INFORMATION FOR SEQ ID NO:81:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

CTTTTTGAAG AGGATTCTCT G 21

(83) INFORMATION FOR SEQ ID NO:82:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

GCCTTTAAAA AATCTGAACA G 21

(84) INFORMATION FOR SEQ ID NO:83:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

ATTACAGTCC TTCACACATC 20

(85) INFORMATION FOR SEQ ID NO:84:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

AGTGTTCACC CTAATAAGCC 20

(86) INFORMATION FOR SEQ ID NO:85:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

CTCCCTGCAC CCTTCCATAA 20

Claims

1. A polynucleotide selected from the group consisting of polynucleotides having a sequence according to SEQ ID NO:64 through SEQ ID NO:85.

2. A method for conducting a PCR procedure comprising using an effective amount of at least one polynucleotide according to claim 1, wherein the polynucleotide is part of a primer pair of polynucleotides selected from the group of polynucleotide pairs consisting of

a) a polynucleotide having the sequence as set forth in SEQ ID NO:64 and a polynucleotide having the sequence as set forth in SEQ ID NO:65;

b) a polynucleotide having the sequence as set forth in SEQ ID NO:66 and a polynucleotide having the sequence as set forth in SEQ ID NO:67;

c) a polynucleotide having the sequence as set forth in SEQ ID NO:68 and a polynucleotide having the sequence as set forth in SEQ ID NO:69;

d) a polynucleotide having the sequence as set forth in SEQ ID NO:70 and a polynucleotide having the sequence as set forth in SEQ ID NO:71;

e) a polynucleotide having the sequence as set forth in SEQ ID NO:72 and a polynucleotide having the sequence as set forth in SEQ ID NO:73.

f) a polynucleotide having the sequence as set forth in SEQ ID NO:74 and a polynucleotide having the sequence as set forth in SEQ ID NO:75;

g) a polynucleotide having the sequence as set forth in SEQ ID NO:76 and a polynucleotide having the sequence as set forth in SEQ ID NO:77;

h) a polynucleotide having the sequence as set forth in SEQ ID NO:78 and a polynucleotide having the sequence as set forth in SEQ ID NO:79;

i) a polynucleotide having the sequence as set forth in SEQ ID NO:80 and a polynucleotide having the sequence as set forth in SEQ ID NO:81;

j) a polynucleotide having the sequence as set forth in SEQ ID NO:82 and a polynucleotide having the sequence as set forth in SEQ ID NO:83; and

i) a polynucleotide having the sequence as set forth in SEQ ID NO:84 and a polynucleotide having the sequence as set forth in SEQ ID NO:85.

3. An assay for measuring the subtle differences in genetic material regarding an added or omitted set of dinucleotide or tetranucleotide repeat polymorphisms wherein said genetic material comprises a sequence characterized by primer pairs 1B-11B, which comprises

a. obtaining polynucleotide segments comprising said repeat polymorphisms in an amount effective for testing,

b. amplifying said segments by a PCR procedure using a pair of oligonucleotide primers capable of amplifying said polymorphism containing segments,

c. resolving the amplified segments using PAGE gel electrophoresis, and

d. comparing the resolved segments by autoradiography to observe the differences in migration patterns due to length variation.

4. An assay according to claim 3, wherein said pair of oligonucleotide primers is selected from the group consisting of oligonucleotides having a sequence according to SEQ ID NO:64 through SEQ ID NO:85.

5. An assay kit for conducting a PCR procedure comprising an effective amount of at least one polynucleotide having a sequence according to claim 1, wherein the polynucleotide is part of a primer pair of polynucleotides selected from the group of polynucleotide pairs consisting of

a) a polynucleotide having the sequence as set forth in SEQ ID NO:64 and a polynucleotide having the sequence as set forth in SEQ ID NO:65;

b) a polynucleotide having the sequence as set forth in SEQ ID NO:66 and a polynucleotide having the sequence as set forth in SEQ ID NO:67;

c) a polynucleotide having the sequence as set forth in SEQ ID NO:68 and a polynucleotide having the sequence as set forth in SEQ ID NO:69;

d) a polynucleotide having the sequence as set forth in SEQ ID NO:70 and a polynucleotide having the sequence as set forth in SEQ ID NO:71; and

e) a polynucleotide having the sequence as set forth in SEQ ID NO:72 and a polynucleotide having the sequence as set forth in SEQ ID NO:73;

f) a polynucleotide having the sequence as set forth in SEQ ID NO:74 and a polynucleotide having the sequence as set forth in SEQ ID NO:75;

g) a polynucleotide having the sequence as set forth in SEQ ID NO:76 and a polynucleotide having the sequence as set forth in SEQ ID NO:77;

h) a polynucleotide having the sequence as set forth in SEQ ID NO:78 and a polynucleotide having the sequence as set forth in SEQ ID NO:79;

i) a polynucleotide having the sequence as set forth in SEQ ID NO:80 and a polynucleotide having the sequence as set forth in SEQ ID NO:81;

j) a polynucleotide having the sequence as set forth in SEQ ID NO:82 and a polynucleotide having the sequence as set forth in SEQ ID NO:83; and

i) a polynucleotide having the sequence as set forth in SEQ ID NO:84 and a polynucleotide having the sequence as set forth in SEQ ID NO:85;

wherein said effective amount of said polynucleotide is in combination with an effective amount of ancillary PCR reagents.

FIGURE 1

AATCTGGGCG ACAAGAGTGA 20

FIGURE 2

ACATCTCCCC TACCGCTATA 20

FIGURE 3

TCCAGCCTCG GAGACAGAAT 20

FIGURE 4

AGTCCTTTCT CCAGAGCAGG T 21

FIGURE 5

GCCAGTGATG CTAAAGGTTG 20

FIGURE 6

AACATACGTG GCTCTATGCA 20

FIGURE 7

AATCTGGGCG ACAAGAGTGA AACTCCGTCA AAAGAAAGAA AGAAAGAGAC 50

AAAGAGAGTT AGAAAGAAAG AAAGAGAGAG AGAGAGAAAG GAAGGAAGGA 100

AGAAAAAGAA AGAAAAAGAA AGAAAGAGAA AGAAAGAAAG AGAAAGAAAG 150

AAAGAAAGAA AGAAAGAAAG AAAGAAAGAA AGAAAGAAAA AGAAAGAAAG 200

AAAGAAAGAA AGAAAGAAAG AAAGAAAGAA AGAAAGAAAG AAAGAAAGGA 250

AGGAAAGAAA GAGCAAGTTA CTATAGCGGT AGGGGAGATG T 291

FIGURE 8

GCCAGTGATG CTAAAGGTTG TATTGCATAT ATACATATAT ATATATATAT 50

ATATATATAT ATATATATAT ATATATATAT ATATATATAT TTTAATTTGA 100

TAGTATTGTG CATAGAGCCA CGTATGTT 128

FIGURE 9

TCCAGCCTCG GAGACAGAAT GAGACTCCAT CAAAAACAAG AAAGAAAGAA 50

AGACAAAGAG AGAAAGAAAG AAAGAAAGAA AGAAAGAAAG AGAGAGAGAG 100

AGAGAGAGAG AGAAAGAAAG AAAGAAAGAA AGAAAGAAAG AAAGAAAGAA 150

AGAAAGAAAG AAAGAAAGAA GGAAAGAAAG AAAGGAAACT AAAATAACTA 200

AATAACTGAG TAGCACCACA CCACCTGCTC TGGAGAAAGG ACT 243

FIGURE 10

TTTCTGGGTG TGTCTGAAT 19

FIGURE 11

ACACAGTTGC TCTAAAGGGT 20

FIGURE 12

CTAGGTTGTA AGCTCCATGA 20

FIGURE 13

TTGAGCACTT ACTCTGTGCC 20

FIGURE 14

AACTCAGAAC AGTGCCTGAC 20

FIGURE 15

ATTTCCCTCA AGGCTCCAGG T 21

FIGURE 16

CTGATCTTGC TCACCTTCGA 20

FIGURE 17

GCGTTTGCTG AAATGAAGGA 20

FIGURE 18

GCAGGTACTT AGTTAGCTAC 20

FIGURE 19

TTACAGTGAG CCAAGGTCGT 20

FIGURE 20

TTTGTCTGGA TAGACTGGAG 20

FIGURE 21

CCATCTTCCT GTGGCTGTA 19

FIGURE 22

CTAATGCAGA GATTTAGGGC 20

FIGURE 23

GTGGTGTAAA GACTGCATAG 20

FIGURE 24

ATGTGACTGA TGTGGGTCAG 20

FIGURE 25

CATCTGCACT CATGCTCCAT 20

FIGURE 26

TCCCAGATCG CTCTACATGA 20

FIGURE 27

CACAGCTTCA GAAGTCACAG 19

FIGURE 28

GAGCAATGTT GCTTAGGATG 20

FIGURE 29

TGGAAGTGTC ACTGGCATGT 20

FIGURE 30

TGTGTCCAGC CTTAGTGTGC A 21

FIGURE 31

TCATCACTTC CAGAATGTGC 20

FIGURE 32

ACTGCCTCAT CCAGTTTCAG 20

FIGURE 33

GAGCAGGCAC TTGTTAGATG 20

FIGURE 34

CCTCTTGGCT CTAACAGCAA 20

FIGURE 35

AGCAAGACCC TGTCTCAAGA 20

FIGURE 36

CAAGGCCCAT CTTCAGTAGA 20

FIGURE 37

CCTTCTCACT CCTTTACTAG 20

FIGURE 38

GAAGACTGAG GAGGTCAGAA 20

FIGURE 39

CTACTGTTCA GAGTCAAAGC 20

FIGURE 40

TGCCCCACAT TAGGATGCAT 20

FIGURE 41

AGGGACACGA ATCAGATCAG 20

FIGURE 42

GTGGTACCTC ATTGTGGCTA 20

FIGURE 43

AGGCATCCTT GTGCTGACAT 20

FIGURE 44

TTTGGCCGAC AGTGGTGTAA 20

FIGURE 45

AGGACCAAAC CATGTCTGTC 20

FIGURE 46

CTGCATCTGA GCATATGGGA 20

FIGURE 47

CATTCAGACT ATGCAGGCTT 20

FIGURE 48

CTGGGACTAC TGGCACATG 19

FIGURE 49

GGCAACGTGG TGAAACCTT 19

FIGURE 50

GGAAGATGGA GTGGCTGTTA 20

FIGURE 51

CTCCAGCCTG GCGAAAGAAT 20

FIGURE 52

GTAAGACTTT TGGAGCCATT 20

FIGURE 53

TTCAGGGAGA ATGAGATGGG 20

FIGURE 54

GACAGAGTGA GACTCCATCT 20

FIGURE 55

GATCCTATCT TCTCAGGAGG 20

FIGURE 56

GAGGTTGCAC TCCAGCCTTT 20

FIGURE 57

ATGCCATGCA GATTAGAAA 19

FIGURE 58

GGAAAGAAAC AGTGAAAGA 19

FIGURE 59

ATCCATCGAC CTCTGGGTTA 20

FIGURE 60

GACCCCACAG CCTATTCAGA 20

FIGURE 61

TTGACTGCTG AACGGCTGCA 20

FIGURE 62

CAGCTGCCCT AGTCAGCAC 19

FIGURE 63

GCTTCCGAGT GCAGGTCACA 20

FIGURE 64

GGGCAACATG GTGAAACCTT 20

FIGURE 65

CCTAGCCTAT ACTTCCTTTC 20

FIGURE 66

GGACCTCGTG AATTACAATC 20

FIGURE 67

ATTTACCTAC CTGTTCATCC 20

FIGURE 68

TTGTGTCAAC TGCTGATATG 20

FIGURE 69

AACCAAAACA TCATTCCCTA 20

FIGURE 70

CGTAAGCGTG CACTATACCC T 21

FIGURE 71

CTGAGGATTC ATCCACCTG 19

FIGURE 72

CCTGAGTAGC TGTTAAGGGA 20

FIGURE 73

GCACATGTAC CCTAGAACTT 20

FIGURE 74

AATCTGAACA GTAATGAAGG 20

FIGURE 75

CATTCTGATA CATTACAGTC 20

FIGURE 76

ATTCCGAGTG ATTTCAGAGA 20

FIGURE 77

TGCTGGTTCA CAGAGCCCTG 20

FIGURE 78

TAGCAGTTCA CAGAGCCCTG 20

FIGURE 79

GTAATTAACA AACCGAGCTG 20

FIGURE 80

AGTATCTGTG CACTGTCTGG 20

FIGURE 81

CTTTTTGAAG AGGATTCTCT G 21

FIGURE 82

GCCTTTAAAA AATCTGAACA G 21

FIGURE 83

ATTACAGTCC TTCACACATC 20

FIGURE 84

AGTGTTCACC CTAATAAGCC 20

FIGURE 85

CTCCCTGCAC CCTTCCATAA 20

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO,A,92 12262 (WASHINGTON UNIVERSITY) 23 July 1992 see the whole document ---	1-5
Y	DATABASE WPI Section Ch, Week 9143, Derwent Publications Ltd., London, GB; Class B04, AN 91-310839 & CA,A,2 009 870 (OREGON HEALTH UNIV) 12 August 1991 see abstract --- -/--	1-5

[X] Further documents are listed in the continuation of box C.

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Date of the actual completion of the international search	Date of mailing of the international search report
15 December 1993	30. 12. 93

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DATABASE WPI Section Ch, Week 9151, Derwent Publications Ltd., London, GB; Class B04, AN 91-369603 & CA,A,2 013 430 (OREGON HEALTH UNIV) 29 September 1991 see abstract	1-5
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